Phase I study of the heparanase inhibitor roneparstat: an innovative approach for multiple myeloma therapy

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SUPPLEMENTUM 1

Roneparstat did show a very good activity when combined with dexamethasone.[1] Roneparstat (60 mg/kg/day for 14 days) and dexamethasone (1 mg/kg/day for 14 days) combination therapy was tested against subcutaneous Myeloma tumor growth in SCID mice (using human MM.1R Myeloma cells) and in Balb/c mice (using murine MPC-11 Myeloma cells), thereby representing drug-resistant and immuno-competent models of Myeloma, respectively. In both settings, the combination therapy significantly inhibited tumor growth more effectively than single agent therapy alone. In the drug-resistant MM.1R model, combination therapy inhibited tumor growth by 80% and in the syngeneic model, combination therapy inhibited tumor growth by 97%. In both cases, assessment of the combination of Roneparstat and dexamethasone revealed a synergistic effect in inhibiting Myeloma tumor growth (Table S1).

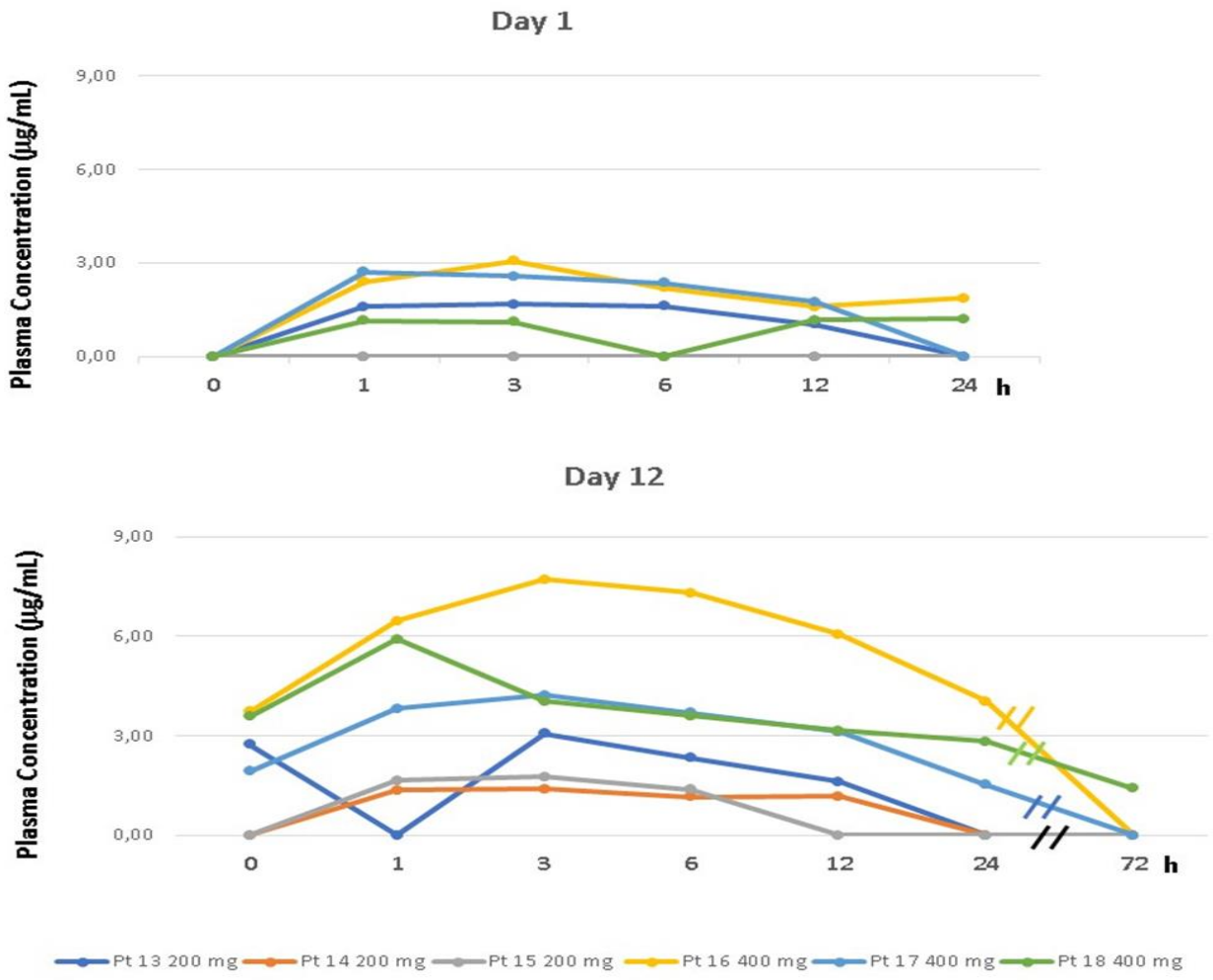
Table S1 - Roneparstat in combination, preclinical models.

Model	**Dose**	**Results**	
		TVI %	**κ chains % inhibition**
MM.1R model, tumour cells injected sc [1] SCID mice	60 mg/kg/day sc injection + dexamethasone 1 mg/kg/day	**80**	-
Syngeneic (MPC-11) model cells injected sc [1] Balb/c mice	60 mg/kg/day sc injection + dexamethasone 1 mg/kg/day	**97**	-
CAG HPSE high cells model Cells intravenously injected in mouse tail veins [2] SCID mice	120 mg/kg/day sc injection + bortezomib 0.5 mg/kg/twice a week	**75%-80%** (Tumour burden reduction by bioluminescence assay)	**70** (only 3/10 animals had detectable levels of serum κ)
CAG HPSE high cells model Cells intravenously injected in mouse tail veins [2] SCID mice	60 mg/kg/day sc injection + melphalan 1 mg/kg/week	**90%-95%** (Tumour burden reduction by bioluminescence assay)	**100**
TVI: tumour volume inhibition; k: human kappa light chain; SCID: Severe Combined Immunodeficiency; sc: subcutaneous			

A preclinical in vivo combination experiment evaluated CAG human Myeloma cells expressing high levels of HPSE (CAG HPSE cells), intravenously injected in mouse tail veins.[2] The CAG HPSE cells are very aggressive, they home to and grow rapidly and almost exclusively in the mouse bones (21 days post-injection), as evidenced by bioluminescence signal, mimicking the late stages of Myeloma. Tumor burden was evaluated by measurement of kappa-levels and bioluminescence after combination treatment with Roneparstat (120 mg/kg/day for 14 days) plus bortezomib (0.5 mg/kg/twice a week for 14 days) (Table S1) or Roneparstat (60 mg/kg/day for 14 days) plus melphalan (1 mg/kg/week for 14 days) (Table S1). Results showed that bortezomib and melphalan efficacy in tumour inhibition was substantially increased when they were combined with Roneparstat. This was evident both from kappa-levels and bioluminescence imaging data; a decrease in the former was always paralleled by a decrease of the latter. This increased efficacy was shown also when Roneparstat was given as sequential therapy after Melphalan.[2]
Positive data of Roneparstat efficacy when combined with Bortezomib or Melphalan are supported by the fact that chemotherapies are known to increase HPSE expression.[3]
Roneparstat also showed activity in tumors other than Myeloma. In particular, an antitumor effect was reported in lymphomas when given alone (60 mg/kd/day) or in combination with Cyclophosphamide, Rituximab or Bevacizumab.[4] Similarly, a strong inhibitory effect was reported in sarcomas models at 60mg/kg/twice daily, especially when combined with 50 mg/kg/day Irinotecan.[5]

Supplementary Figure S1 reports the plasma profiles of individual patients receiving 200 mg and 400 mg. Plasma levels of Roneparstat were quantifiable at cycle 1 at the two highest dose levels after single (day 1) and repeated (day 12) dosing using fluorescent probe assay (Heparin Red).[6]
Systemic exposure appears reproducible, linear but slightly over-proportional with the dose. These data seem to reflect preclinical PK (rat & monkey).[7]
With all the caution due to the translation from preclinical to clinical findings, the reported plasma levels are coherent with preclinical data showing that the inhibitory ability of the drug is in the nanomolar range, with an IC_{50} value corresponding to 0,06 µg/mL.[8] Moreover, a non-clinically significant increase of aPTT and of TT was detected in one and two patients receiving 400 mg, respectively, thus further showing patient exposure to the drug.
The effect on coagulation occurring in the patients who received 400 mg suggested this as the highest dose level to be explored. In combination phase I/II studies the dose identified to be used will be 300-400 mg/day.

Figure S1 - Day 1 and Day 12 plasma profiles in patients treated with Roneparstat 200 mg (3 patients) and 400 mg (3 patients).



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